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Principles of Viral Pathogenesis

Minireview

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Patho is derived from the Greek πάθος, meaning suffering or disease, and genesis from the Greek γένεσις, which translates "to come into being or origin." Viral pathogenesis, then, is defined as "how viruses produce disease in the host." The portrait of viral pathogenesis is the sum of functions through which a virus causes disease (virulence) and the host resists or is susceptible. Despite the avalanche of knowledge concerning viral gene structure and function, and the ability to manipulate both cultured cells and animal models genetically, our understanding of how viruses cause disease is scanty. As an example, for poliovirus, despite the wealth of information on its molecular structure, control of gene regulation including the details of its replication, transcription, translation, and assembly, the solving of its three-dimensional structure, the discovery of its receptor, and the availability of a transgenic model, our knowledge of how poliovirus actually causes poliomyelitis is limited. Considering the number of persons infected with the virus, few develop poliomyelitis. In 1955, when an outbreak of poliomyelitis followed the use of a formaldehyde-inactivated poliovirus vaccine, of the 120,000 children inoculated with the improperly inactivated lots of polio type 1, approximately 60,000 (or 50%) were susceptible to the infection in that they had no evidence of preexisting immunity. Yet, of those 60,000, only between 600 and 1500 became infected, as evident by minor clinical findings and/or fecal excretion of virus, and fewer than 0.1% (or 60 patients) developed paralytic polio. Still unknown is why fewer than 1% of people who encounter poliovirus for the first time get the disease, compared to greater than 99% who become ill after infection by measles virus. Thus, the determinants that control viral virulence or host susceptibility (or resistance) in the natural host are far from being understood in terms of molecules or genes involved.

Yet, for poliovirus, the pathologic consequences of acute and limited infection in vivo are rather easy to follow. Polioviruses reach the central nervous system anterior horn cells in susceptible individuals during the first week of infection. These cells are destroyed within hours and, if enough are affected, muscles become paralyzed as the characteristic disease progresses. Why poliovirus has such a high affinity for anterior horn neurons but not other types of neurons in the central nervous system is not understood, nor is it known why, when most cells presumably express poliovirus receptors, only neuronal cells and perhaps cells of the lymphoreticular system become infected.

This ignorance of pathogenic mechanisms is not unique to poliovirus. Equally obscure is how HIV causes dementia and immunosuppression and why measles virus promotes generalized immunosuppression, and so on.

Much of our existing knowledge of viral pathogenesis has come from experimental studies using animal models of virus–host interactions. Often the principles derived have provided universal concepts that proved applicable to human diseases. Perhaps the best examples are reovirus (reviewed by Nibert et al., 1991) and lymphocytic choriomeningitis virus (LCMV; reviewed by Borrow and Oldstone, 1996) infections of the mouse. Mammalian reoviruses are non-enveloped viruses that have a genome consisting of 10 segments of double-stranded RNA. Although this virus has not yet been linked to any severe disease of humans, manipulation of reovirus genes in mice successfully illustrates the overall strategies viruses use in binding to and entering primary cells and in replicating, growing, and spreading to secondary target organs in vivo. Such studies indicate that reoviruses can be considered as a general model for many other viruses that undoubtedly use similar pathways and principles. The beauty of this model is that the viral genes and their products can be defined at each step of viral binding to cells, entry, replication, and spread in the animal host, as can their interactions with the host's immune system.

LCMV is a negative-strand, ambisense virus having two RNA segments. Analysis of the parameters defining acute LCMV infection has led to the initial observations that T lymphocytes are a major controlling factor of many viral (and other infectious) diseases, MHC compatibility is required for activation of cytotoxic T lymphocytes (CTL) and their recognition of infected cells (an observation that was rewarded with this year's Nobel Prize in Medicine and Physiology) and MHC diversity is favorable in the battle against infectious agents (Zinkernagel and Doherty, 1979). Similarly, a number of the defining pathogenic events associated with DNA and RNA viruses that persist in humans have also been established and/or clarified from the study of LCMV infection in its natural murine host. Examples include the recognition that viral persistence most often requires replication of the virus in cells (lymphocytes, dendritic macrophages) of the immune system and that antiviral antibodies interact with the virus or its antigens to form virus–antibody immune complexes. Not only are these immune complexes markers of persistent infection but they also cause the vasculitis (deposition of immune complexes in arteries), glomerulonephritis (deposition of immune complexes in the renal glomeruli), and mental confusion (deposition of immune complexes in the brain's choroid plexus) that accompany many, if not all, persistent infections.

Unlike the relatively short-lived acute infections, persistent virus infections offer an opportunity for prolonged pathologic consequences. But how do viruses persist? What are the molecular determinants, how do

they damage tissue, and what kind of disease(s), in addition to immune complexes, can they cause?

Viruses that persist must first devise a strategy to remain within cells for a prolonged period of time without disturbing the transcription or translation of the genes necessary for the infected cells' survival. Also, such viruses must not alter lysosomal or plasma membranes or cytoskeletal structures of the infected cells. Second, viruses must escape immune surveillance. Since the CTL is the major player in discriminating self (host antigens) from foreign (viral antigens), viruses need to evade recognition by CTL. Most often this occurs by interference with antigen presentation, MHC restriction, CTL activation, and/or CTL activity (reviewed by Furth et al., 1996). For example, human herpes simplex virus (HSV) expresses an immediate-early protein, IPC-47, that blocks presentation of viral peptides to MHC class I–restricted CTL. IPC-47 binds TAP, a protein transporter normally responsible for translocating the cytosolic peptide to the endoplasmic reticulum (ER) where MHC molecules are synthesized (Furth et al., 1996; Hill et al., 1995). Human cytomegalovirus (HCMV) uses a series of different genes, unique short (US) 2 and 11, to rapidly degrade the MHC complex, presumably by shunting the MHC complex back out into the cytosol for degradation by proteosomes (Furth et al., 1996; Wiertz et al., 1996). Although HSV and HCMV studies are elegant biochemically, the role of these viral genes in persistent infection should be interpreted cautiously until there is evidence that they reproduce their in vitro effect in vivo and in cells where they usually persist in (HSV/neurons, HCMV/monocyte-macrophages, and lymphocytes). The adenovirus A2 E3 gene complex contains and encodes a gp19 molecule, the carboxy terminus of which contains a sequence motif that binds to MHC class I molecules and retains them in the ER (Furth et al., 1996; Jackson et al., 1990). Disturbances in antigen presentation by the adenovirus E3 gene complex have been observed both in vitro (Furth et al., 1996; Jackson et al., 1990) and in vivo (Efrat et al., 1995). LCMV persists in vivo by two distinct mechanisms. First, mice infected neonatally or in utero with LCMV become persistently infected for life because such animals are unable to mount an effective antiviral CTL response as virus replicates in the thymus and specifically deletes (negative selection) LCMV-reactive T cells (King et al., 1992; Pircher et al., 1989).

Second, in such persistently infected mice, LCMV variants are generated and selected for replication in lymphocytes and macrophages (Ahmed and Oldstone, 1988). Unlike the wild-type parental virus, these variants, when inoculated into immunocompetent adult mice cause persistent infection. In contrast, similar inoculation of the parental virus generates LCMV-specific CTL and clears the virus so that neither persistent infection nor immunosuppression occurs. Genetic and biochemical analysis to compare the parental Armstrong-strain (ARM) virus and its progeny immunosuppressive variants (like Clone 13) revealed a Phe (wild-type) to Leu (immunosuppressive variant) change at amino acid 260 of the viral glycoprotein (GP) as a marker for both classes of virus. Although several mutations occur in the genome, this one is always associated with virus-induced immunosuppression. As early as three to five weeks post-infection with the wild-type strain, viruses with the Phe to Leu change are found in murine recipients' spleens, lymph nodes, and macrophages. In contrast, the Phe marker does not change in infected neurons even at one year post-infection. Selective competition both in vitro and in vivo (Dockter et al., 1996) favors the wild-type strain over Clone 13 for neuronal replication. However, more efficient replication of Clone 13 than of the wild-type in antigen presenting cells and splenic lymphocytes suggests that unique cellular transcription factors are likely to play a role in selection of one virus species over another and illustrates the need to study interactions between each virus and the particular differentiated cell(s) it infects rather than a conveniently available cell in the laboratory.

Biologically, adult mice persistently infected with the immunosuppressive variant exhibit a generalized and not just a LCMV-specific immunodeficiency, since they fail to mount efficient immune responses to other viruses, parasites, or tumors. The mechanism involved is preferred replication of this immunosuppressive variant in follicular interdigitating dendritic (professional antigen-presenting) cells and in the spleen and lymph nodes, as well as persistent infection of T lymphocytes and macrophages. By contrast, the parental ARM strain replicates preferentially in F4/80$^+$ macrophages in the red pulp of the spleen (Borrow et al., 1995). Hence, the tropism of the immunosuppressive LCMV variant in CD4$^+$ T cells and macrophages, involvement of professional antigen-presenting dendritic cells, and the resultant generalized immunosuppression closely mimic events typical of HIV infection. The causes of this immunosuppression is destruction of the virus-infected dendritic cells by CD8$^+$ LCMV CTL (Borrow et al., 1995), the same immunopathologic process postulated for HIV infection (Hayes et al., 1996).

What is the consequence to a differentiated cell once occupied by a virus that persists? In one example, inoculation of newborn C3H/St mice with LCMV ARM leads to persistent infection during which the virus replicates in growth hormone (GH)-producing cells of the pituitary gland's anterior lobe (Borrow and Oldstone, 1996). Although no structural abnormality of such GH-producing cells is evident in vivo, the transcription of GH decreases approximately 16-fold with a resultant 5-fold reduction in GH mRNA and a 2-fold reduction in the synthesis of GH. The outcome is that the infected mouse fails to grow and develop, becomes hypoglycemic, and consequently dies within 30 days.

How does this GH deficiency occur? What roles do viral genes play? Experiments to answer these questions were based on the fact that LCMV ARM causes GH disease in C3H/St mice, but other LCMV strains like WE do not. LCMV has a bisegmented negative-stranded genome consisting of two species of single-stranded RNA, designated L (large) and S (small). S RNA encodes the virus GP and nucleoprotein (NP), while L RNA encodes the viral polymerase (L) and a small protein (Z) with an as yet unknown function but containing a zinc-binding motif. Reassortants between LCMV ARM Clone 53b (GH$^+$) and WE Clone 54 (GH^{nil}) map the GH disease syndrome to genes encoded on the S RNA, i.e., the GP or NP. To focus on the role of the viral GP and/or NP

genes, advantage was taken of observations from RNA viruses in which the high frequency of mutations occurring during replication created heterogeneous mixtures of genetically closely-related genomes. Clones picked from a WE 54 (GH^{nil}) mixture of such quasispecies revealed that over 95% showed the same phenotype as the parental WE 54 clone in that they failed to cause GH disease. However, 5% of the isolated clones caused GH disease. Reassortants between a WE 54 clone that caused GH disease and those that did not mapped the defect to the S RNA. Sequencing showed that the NP structure was conserved among the WE isolates but that mutations in the GP gene product at amino acid 153 caused a change from Ser to Phe. Interestingly this mutation occurred in the part of GP that has been implicated in binding to the LCMV receptor, suggesting that the defect in GP (WE 54 [GH^{nil}]) may be at the level of receptor binding. To determine whether the NP gene from WE 54 (GH^{nil}) and ARM (GH^{+}), once inside GH-forming cells, participated in the GH deficiency, two approaches were used. In the first, vaccinia recombinants were made that encoded LCMV ARM GP, NP, or non-LCMV genes. Such studies showed that LCMV NP, but not GP or the non-LCMV genes, disordered GH transcription in appropriately transfected cells. The second approach was construction of a transgenic mouse model in which the GH promoter expressed LCMV ARM NP. Under these conditions, the transgenic mice displayed many manifestations of the GH deficiency syndrome. In contrast, expression of non-NP genes had no effect. Hence, both the GP and NP gene products are associated with GH disease. GP is important for the binding and entry of a virus into the appropriate cell (in C3H/St mice, anterior lobe cells of the pituitary gland, which makes GH) and NP for affecting transcription of GH. But how does the viral NP influence transcription? The effect of LCMV infection on GH synthesis in a pituitary cell line displayed findings similar to those observed in vivo, i.e., five-fold decrease in specific GH mRNA transcription with no effect on prolactin (PL) mRNA transcription. With nuclear run-on assays, that defect was determined to occur at the level of initiation of transcription. Other studies, using the GH promoter or PL promoter fused to an indicator gene like CAT, placed the virus' effect at the level of the promoter; subsequently, deletion mutants of the GH promoter mapped the defect to the area on the promoter that bound the GHF1 transcription factor. In agreement, levels of GHF1 transcription factor were significantly reduced in LCMV-infected pituitary cells compared to levels in uninfected cells. Hence, once inside the cell, the viral NP expressed in the cytosol interferes with GH synthesis by decreasing the amount of GHF1 able to bind to the GH promoter and a selective rather than general inhibition of transcription occurs. Because viruses disorder the function of the GH-producing cell without killing it, an opportunity is available to reverse the disease by use of antiviral therapy (de la Torre and Oldstone, 1992). Accordingly, ribavirin, an antiviral agent that is effective against several arenaviruses, the family to which LCMV belongs, not only clears virus from infected pituitary cells, but restores the GH-synthesizing complex to normal in treated cells.

Studies from this and other models highlight a number of general principles. First, most viruses that persist probably do so by impairing either antigen presentation or CTL recognition. Hence, studies of persistent infections are likely to prove of value in dissecting the biology of both these pathways. Second, it is possible in vivo and in vitro to manipulate viral genes to determine which ones are involved in specific diseases and how they produce their effects. This strategy has been used successfully with several viruses. Unfortunately, space limitations in this review preclude discussion of similar analyses for host gene expression, gene knockout mice, or polymorphic microsatellites distributed along chromosomes used to map effects by host genes. Third, it is now clear that, in addition to causing the GH syndrome presented here, viruses that persist can alter the function of the other differentiated cells in vivo including neurons, other endocrine cells, and cells of the immune system. Recent evidence indicates that, in several instances, antiviral therapies can restore normalcy and correct such diseases. Since we humans are continually bathed in a sea of microbes, yet harmed by relatively few, persistence by viruses is likely to be a common event, and viruses are likely to be responsible for a wide variety of illnesses whose cause is currently unknown.

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